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(54) **Use of somatostatin.**

(57) The invention provides the use of somatostatin or a somatostatin agonist for the manufacture of a medicament for use in a method of inhibiting proliferation of bile duct epithelium.

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This invention r lates to a method of inhibiting the growth of benign or malignant tumor.

Cholangiocarcinoma refers to any malignancy originating in the intra- or extrahepatic bile ducts. These tumors probably represent 10-15% of primary hepatobiliary cancers, and their incidence in the United States is estimated at up to 3,000 per year (see Yeo et al., Surg. Clin. North Amer. 70:1429(1990) and Vogt et al., Oncology 2:37(1988)). The prognosis for these tumors is universally dismal, with a majority of patients dying within six months to one year of diagnosis (see Imrie et al., Wright's Liver and Biliary Diseases, Vol 2, 3rd ed., page 1516 (1992)).

Cholangiocarcinomas are notoriously difficult to diagnose. The diagnosis is usually performed by non-invasive imaging studies (e.g. ultrasound or CT scan) with an overall accuracy of only about 50%. Positive histology is required for absolute diagnosis, and this is most effectively performed by fine needle aspiration biopsy (see Desa et al. Gut 32(10):1188(1991) and Bedrossian et al., Arch. Patho. Lab. Med. 113(11):1225(1989)). However, by the time this diagnosis is made, the disease has commonly reached the point where curative surgical therapy is impossible (see Yeo et al., Cholangiocarcinoma (Review) Surg. Clin. North Amer. 70:1429(1990) and Miyasaki et al., Surg. Ther. 56:443 (1987)).

Surgical resection presently provides the best hope for cure. However, only ten percent of patients with cholangiocarcinomas are surgically treatable because of the location of their tumors (see Nargoney et al., Sem. Oncol. 15:106(1988)). Even after resection, twenty-five percent of these patients will still have residual tumors, and the five year survival rate for resected patients is only thirteen percent with an operative mortality up to twelve percent (see Yeo et al., Surg. Clin. North Amer. 70:1429(1990) and Boerma et al., Surgery 215:31(1990)).

The possibility of liver transplantation has also been attempted as a potential treatment modality. This treatment, however, has generally been unsuccessful since the recurrence of cholangiocarcinomas is virtually 100%. The survivorship, therefore, appears to be no better than that of the untreated patient (see Penn et al., Surgery 110:726(1991) and Jenkins et al., Cancer Chemother. Pharmac. 23:S104(1989)). Many liver transplant centres have abandoned transplantation of patients with cholangiocarcinomas. Other centres consider transplantations for cholangiocarcinoma to be experimental in nature. In some centres, protocols employing adjuvant chemotherapy and/or radiation therapy in conjunction with transplantation are under investigation.

Non-surgical therapy of cholangiocarcinomas has been limited to patients who are found to be unresectable on the basis of either preoperative imaging or surgical exploration. Current modalities generally include variations of radiation therapy (e.g. external radiation with or without chemotherapy or the combination of external radiation or intraluminal brachytherapy). Although external irradiation with or without chemotherapy may lead to prolonged survival in some patients, the side effects of these forms of therapy are often severe as the nearby liver, gut, and spinal cord are irradiated as well (see Gunderson et al, "Principles and Practice of Radiation Oncology", page 985, 1992). Palliative stenting helps to relieve the symptoms of cholangitis, but sepsis may occur in up to 88% of percutaneous stents (see Lokich et al, J. Clin Oncol. 5:969(1987)).

The present invention relates to the use of somatostatin or a somatostatin agonist for the manufacture of a medicament for use in a method of inhibiting proliferation of bile duct epithelium. This method of treatment may be used to inhibit benign or malignant proliferation and includes the step of contacting the proliferating epithelial cells with an effective amount of somatostatin or a somatostatin agonist. It is preferred that the contacting step be effected parenterally, e.g. by administration to the proliferation site in a subject intravenously, subcutaneously, by implantation (e.g. near the liver) or by perfusion (e.g. of the liver). Drug delivery by implantation is well known in the art (see for example US-A-4675189 and US-A-4767628.

The term cholangiocarcinoma as used herein also covers any malignant proliferation of bile duct epithelium.

The following abbreviations are also used herein:

β-Nal = β-naphthylalanine
β-Pal = β-pyridylalanine
hArg(Bu) = N-guanidino-(butyl)-homoarginine
hArg(Et)$_2$ = N,N'-guanidino-(diethyl)-homoarginine
hArg(CH$_2$CF$_3$)$_2$ = N,N'-guanidino-bis-(2,2,2-trifluoroethyl)-homoarginine
hArg(CH$_3$, hexyl) = N,N'-guanidino-(methyl,hexyl)homoarginine
Lys(Me) = N$^\epsilon$-methyl-lysine
Lys(iPr) = N$^\epsilon$-isopropyl-lysine
AmPhe = aminomethylphenyl-alanine
AChxAla = aminocyclohexylalanine
Abu = α-aminobutyric acid
Tpo = 4-thiaproline
MeLeu = N-methyl-leucine

Orn = ornithine
Nle = norleucine
Nva = norvaline
Trp(Br) = 5-bromo-tryptophan
Trp(F) = 5-fluoro-tryptophan
Trp(NO_2) = 5-nitro-tryptophan
Gaba = γ-aminobutyric acid
Bmp = β-mercaptopropionyl
Ac = acetyl
Pen = penicillamine

The dosage of somatostatin or the somatostatin analog required will naturally depend upon the condition being treated, the route of administration chosen, and the specific activity of the compound used, and as is usual in the medical field will ultimately be decided by the attending physician or veterinarian.

While it is possible for somatostatin or the somatostatin analog to be administered as a pure or substantially pure compound, it is preferable to present it as a pharmaceutical formulation or preparation.

The medicaments to be produced according to the present invention, for both human and animal administration, will generally comprise one of the octapeptide analogs described below, together with one or more pharmaceutically acceptable carriers therefor, and optionally other therapeutic ingredients.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient(s) of the formulation (and preferably, capable of stabilizing peptides) and not deleterious to the subject to be treated. Desirably, the formulation should not include oxidizing agents or other substances with which peptides are known to be incompatible. For example, somatostatin analogs in the cyclized form are oxidized; thus, the presence of reducing agents as excipients could lead to an opening of the cystine disulfur bridge. On the other hand, highly oxidative conditions can lead to the formation of cysteine sulfoxide and to the oxidation of tryptophan. Consequently, it is important to select the excipient carefully . Moreover, pH is another key factor and it is preferable to buffer the product under slightly acidic conditions (pH 5 to 6).

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients.

In general, the formulations for tablets or powders are prepared by uniformly and intimately blending the active ingredient with finely divided solid carriers, and then, if necessary as in the case of tablets, forming the product into the desired shape and size.

Formulations suitable for intravenous administration, on the other hand, conveniently comprise sterile aqueous solutions of the active ingredient(s). Preferably, the solutions are isotonic with the blood of the subject to be treated. Such formulations may be conveniently prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering said solution sterile. The formulation may be presented in unit or multi-dose containers, for example, sealed ampoules or vials.

Somatostatin (somatotropin release inhibiting factor, or SRIF) is an inhibitor of secretion of the growth hormone and was originally isolated from the hypothalamus (see Brazeau et al., Science 179:77(1973)). Somatostatin has a broad spectrum of biological effects, participates in a high number of biological processes and, in the majority of cases, plays the role of an inhibitory factor (e.g. it inhibits the release of prolactin, insulin, glucagon, gastrin, secretin, and cholecystokinin - see Reichlin, "Somatostatin", N. Eng. J. Ned. 309:1495 and 1556(1983)).

Native somatostatin has a very short duration of effect *in vivo* since it is rapidly inactivated by endo- and exopeptidase. Many novel analogs have been prepared in order to enhance the duration of effect, biological activity, and selectivity of this hormone. Such analogs are called somatostatin agonists herein.

Somatostatin agonists which can be used according to the present invention include, but are not limited to, those covered by the formulae or specifically recited in the following publications:
EP-A-505680; Van Binst et al. Peptide Research 5:8(1992); Horvath et al. "Conformations of Somatostatin Analogs Having Antitumor Activity", 22nd European Peptide Symposium, September 13-19, 1992, Interlaken, Switzerland; WO-A-91/09056; EP-A-363589; US-A-4904642; US-A-4871717; US-A-4853371; US-A-4725577; US-A-4684620; US-A-4650787; US-A-4603120; US-A-4585755; EP-A-203031; US-A-4522813; US-A-4486415; US-A-4485101; US-A-4435385; US-A-4395403; US-A-4369179; US-A-4360516; US-A-4358439; US-A-4328214; US-A-4316890; US-A-4310518; US-A-4291022; US-A-4238481; US-A-4235886; US-A-4224190; US-A-4211693; US-A-4190648; US-A-4146612; and US-A-4133782.

Preferred somatostatin agonists include, but are not limited to, the following somatostatin compounds which are disclosed in the above-mentioned references:

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;
H-D-β-Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr;
H-Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr;
H-Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr;
H-Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-threoninol;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;
H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;
Ac-D-Phe-Lys*-Tyr-D-Trp-Lys-Val-Asp-Thr-NH_2 (with an amide bridge formed between Lys* and Asp);
Ac-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Et)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-L-hArg(Et)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;
Ac-L-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NHEt;
Ac-hArg(CH$_3$,hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
H-hArg(hexyl)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;
Ac-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH$_2$;
Propionyl-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH$_2$;
Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg(Et)$_2$-NH$_2$;
Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-D-hArg(CH$_2$CF$_3$)$_2$-D-hArg(CH$_2$CF$_3$)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH$_2$;
Ac-D-hArg(Et)$_2$-D-hArg(Et)$_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH$_2$;
Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH$_2$;
Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$;
Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH$_2$;
Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH$_2$;
Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH$_2$;
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH$_2$;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH$_2$;
H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$;
Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH$_2$;
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH$_2$;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH$_2$;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;
H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;
Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;
H-D-Phe-Cys-β-Nal-D-Trp-Lys-Val-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2;
cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);
cyclo(Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);
cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);
cyclo(Pro-Tyr-D-Trp-Lys-Thr-Phe);
cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe);
cyclo(Pro-Phe-L-Trp-Lys-Thr-Phe);
cyclo(Pro-Phe-D-Trp(F)-Lys-Thr-Phe);
cyclo(Pro-Phe-Trp(F)-Lys-Thr-Phe);
cyclo(Pro-Phe-D-Trp-Lys-Ser-Phe);
cyclo(Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);
cyclo(D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);
cyclo(D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);
cyclo(D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);
cyclo(D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);
cyclo(Pro-Tyr-D-Trp-t-4-AChxAla-Thr-Phe);
cyclo(Pro-Phe-D-Trp-t-4-AChxAla-Thr-Phe);
cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);
cyclo(N-Me-Ala-Tyr-D-Trp-t-4-AChxAla-Thr-Phe);
cyclo(Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo(Pro-Phe-D-Trp-4-Amphe-Thr-Phe);
cyclo(N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);
cyclo(Asn-Phe-D-Trp-Lys-Thr-Phe);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-NH-$(CH_2)_4$CO);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-β-Ala);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);

cyclo(Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp(NO_2)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Tpo-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-MeLeu-Cys)-OH;
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);
cyclo(Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH(CH_2)$_3$-CO);
cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba); and
cyclo(Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba).

Also preferred somatostatin agonists for the invention are the compounds of the following formula:

$$\begin{array}{l} R_1 \\ \quad \diagdown \\ \qquad A_1\text{-}A_2\text{-}A_3\text{-D-Trp-Lys-}A_6\text{-}A_7\text{-}A_8\text{-}R_3 \\ \quad \diagup \\ R_2 \end{array}$$

(wherein A_1 is a D- or L-isomer of β-Nal, Trp, β-Pal, Phe, substituted Phe, or is deleted; A_2 and A_7 independently are Cys, Asp, or Lys, and are covalently linked either via a disulphide bridge or an amide bridge; A_3 is β-Nal, Phe, or o-, m-, or p-substituted X-Phe (where X is a halogen, OH, NH_2, NO_2 or C_{1-3} alkyl); A_6 is Val, Thr, Ser, Ala, Phe, β-Nal, Abu, Ile, Nle, or Nva; and A_8 is Phe, Thr, Tyr, Trp, Ser, β-Nal, -NHCH(CH[CH_3]OH)-CH_2- or is deleted; each R_1 and R_2 independently is H, lower acyl or lower alkyl; and R_3 is OH or NH_2; with the provisos that when one of A_2 and A_7 is Cys, the other is also Cys, and that when neither of A_2 and A_7 is Cys, A_2 is different from A_7).

Where a group is referred to herein as being lower alkyl or lower acyl, it will conveniently have a carbon atom content of up to 12, eg 1 to 6.

Particularly preferred somatostatin agonists of this formula to be used according to the invention include:

N-Me-D-Phe-Cys-Tyr-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and
D-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH_2

The use of linear somatostatin agonists of the following formula is also within the scope of the invention:

$$\begin{array}{l} R_1 \\ \quad\diagdown \\ \qquad A^1\text{-}A^2\text{-}A^3\text{-D-Trp-Lys-}A^6\text{-}A^7\text{-}A^8\text{-}R_3 \\ \quad\diagup \\ R_2 \end{array}$$

(wherein A^1 is a D- or L- isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, β-Nal, β-pal, Trp, Phe, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe or o-X-Phe (wherein X is CH_3 Cl, Br, F, OH, OCH_3 or NO_2); A^2 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe (wherein X is CH_{3-1} Cl, Br, F, OH, OCH_3 or NO_2); A^3 is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe (wherein X is CH_3 Cl, Br, F, OH, OCH_3 or NO_2); A^6 is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser; A^7 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe (wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2); A^8 is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, -NH-CH(CH[CH_3]OH)-CH_2-, Ser, Phe, β-Nal , pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe (wherein X is CH_3 Cl, Br, F, OH, OCH_3 or NO_2); each R_1 and R_2 independently is H, lower acyl or lower alkyl; and R_3 is OH or NH_2; with the provisos that at least one of A^1 and A^8 and one of A^2 and A^7 must be an aromatic amino acid and that A^1, A^2, A^7 and A^8 are not all aromatic amino acids).

Particularly preferred linear agonists to be used according to this invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2;
H-D-Phe-p-NO_2-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;
H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;
H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2;
H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;
H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2; and
H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-β-D-Nal-NH_2

If desired, one or more hydrophilic moieties (e.g. a sugar derivative or an ascorbic acid derivative) can be attached to the somatostatin agonist (see WO-A-88/02756 and EP-A-329295). An example of a particularly preferred somatostatin agonist which contains hydrophilic moieties is:

N^α-(α-glucosyl(1-4)-deoxyfructosyl)-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-threoninol.

It should be noted that, unless indicated otherwise, for all the somatostatin agonists described herein, each amino acid residue (e. g. Lys and A^1) has the structure -NH-C(R)H-CO-, in which R is the side chain. Lines between amino acid residues represent peptide bonds which join the amino acids. Also, where the amino acid residue is optically active, it is the L-form configuration that is intended unless D-form is expressly designated. A disulphide bridge is formed between two Cys residues; however, it is not shown.

The synthesis of one exemplary octapeptide somatostatin agonist with an amide C-terminus is described hereafter by way of general illustration. Other such octapeptides as are used in the invention can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in this field, of the synthetic method disclosed herein.

The first step in the synthesis of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 was preparation of the intermediate, tert-butyloxycarbonyl("Boc")-D-β-Nal-S-p-methylbenzyl-Cys-Tyr-D-Trp-ε-N-benzyloxycarbonyl-Lys-Val-S-p-methylbenzyl-Cys-O-benzyl-Thr-benzyhydrylamine resin, as follows.

Benzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) in the chloride ion form was placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride wash; (b) treatment with 33% trifluoroacetic acid in methylene chloride (two times, for 1 min. and 25 min. each); (c) methylene chloride wash; (d) ethanol wash; (e) methylene chloride; (f) treatment with 10% triethylamine in chloroform.

The neutralized resin was then stirred with Boc-O-benzyl-threonine and diisopropylcarbodiimide (1.5

mmole each) in methylene chloride for 1 hr. and the resulting amino acid resin was cycled through steps (a) to (f) in the above program. The following amino acids (1.5 mmole) were then coupled successively by the same procedure: Boc-S-methylbenzyl-Cys, Boc-Val, Boc-∊-N-benzyloxycarbonyl-Lys, Boc-D-Trp, Boc-Tyr, Boc-S-p-methylbenzyl-Cys, and Boc-D-β-Nal.

Thereafter, the resin was washed with methylene chloride, dried, and then mixed with anisole (4 ml) and anhydrous hydrogen fluoride (36 ml) at 0°C and stirred for 45 min. (Alternatively one can also use thioanisole, trifluoroacetic acid, and trifluoromethane sulphonic acid at a ratio of 1:90:9, for 6 hr). Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen, the peptide and resin were treated with ether, and the resin and peptide were removed by filtration and washed with ether. The crude peptide thus obtained was then dissolved in 800 ml of 90% acetic acid to which was added I_2 in methanol until a permanent brown color appeared. The solution was then stirred for 1 hr. before removing the solvent under reduced pressure. The resulting oil was dissolved in a minimum volume of 50% acetic acid and placed on a Sephadex G-25 column (2.5 x 100 mm) which was then eluted with 50 % acetic acid. Fractions containing a major component as shown by UV absorption and thin layer chromatography ("TLC") were then pooled, evaporated to a small volume, and applied to a column (2.5 x 50 cm) of Whatman LRP-1 octadecylsilane (15-20 μM).

The column was eluted with a linear gradient of 10-50% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions were examined by TLC and analytical high performance liquid chromatography ("HPLC") and pooled to give maximum purity. The trifluoracetic acid (TFA) salt was prepared. Repeated lyophilization of the solution from water gave 170 mg of the product as a white, fluffy powder.

The product was found to be homogeneous by both HPLC and TLC. Amino acid analysis of an acid hydrolysate confirmed the composition of the octapeptide.

Also synthesized according to a method similar to that described above was among others, H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH_2, another octapeptide analog of somatostatin with an amide C-terminus which can be employed for the treatment of cholangiocarcinoma.

The methods for synthesizing somatostatin agonists are well documented and are within the ability of a person of ordinary skill in the art. For example, synthesis of the peptide H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-threoninol (octreotide) described above can be achieved by following the protocol set forth in Example 1A of US-A-4395403.

Assays for Determining Anti-Proliferative activity

1. Tumor system

The human cholangiocarcinoma cell line SK-ChA-1 was grown from malignant ascites of a patient with primary adenocarcinoma of the extrahepatic biliary tree. (see Knuth et al., J. of Hepatology 1:579(1985)).

2. Assay system

SK-ChA-1 cells were plated (100 μl) into 96-well culture plates (Falcon) at a density of 50,000 cells/ml in Dulbecco's modified Eagle's medium (DMEM/HAMSF12 (50:50)) with 2% foetal calf serum (FCS, Hyclone) and antibiotics (penicillin 100IU/ml, streptomycin 100 μg/ml and amphotericin B 2.5 μg/ml). After 18 hours, the medium was replaced with fresh medium with or without the two test drugs, natural somatostatin-14 and the somatostatin agonist octreotide. The experiments were performed in triplicate. Wells without the addition of any test drug served as normal controls. All media, with the appropriate concentration of test drug, were changed every two days.

The amount of viable cells was assayed every two days using 3-(4,5-dimethylazol-2-yl)-2,5-diphenyl-tetrazolium (MTT) accordirig to method published by Tada, J. Immunol. Meth. 93:157(1986). Optical density was read by a 96-well automatic microplate reader (Molecular Devices) at a test wavelength of 570 nm and a reference wavelength of 630 nm. An experiment had validated the correlation of the MTT assay (r=0.975, p=0.004) with a wide range of cell concentrations (3,000 to 330,000 cells/ml) counted with the hemocytometer. Cell proliferation curves with respect to time and test drug concentration were plotted with control wells as the baseline for comparison.

Two experiments were performed using this assay. The first experiment was performed at drug concentrations of 10^{-5} M to 10^{-8} M (in one log increments) and cell proliferation was assayed every two days until day 6. The second experiment was performed at conc ntrations of 10^{-5} to 10^{-10} M (in one log increments) and assayed every two days until day 8.

3. Results

Table I shows the results of the first experiment, while Table II shows the results of the second experiment.

Table I

Drug Concentration (M)		Percent Protection, Day 8
Somatostatin	1×10^{-5}	94.9
	1×10^{-6}	66.8
	1×10^{-7}	89.9
Octreotide	1×10^{-5}	79.1
	1×10^{-6}	92.2
	1×10^{-7}	78.4
	1×10^{-8}	61.0

Table II

Drug Concentration (M)		Percent Protection, Day 4
Somatostatin	1×10^{-5}	54.3
	1×10^{-6}	47.5
	1×10^{-7}	42.6
	1×10^{-8}	50.2
	1×10^{-9}	50.4
	1×10^{-10}	62.3
Octreotide	1×10^{-5}	62.6
	1×10^{-6}	52.9
	1×10^{-7}	44.4
	1×10^{-8}	46.8
	1×10^{-9}	61.7
	1×10^{-10}	50.4

The accompanying figures illustrate the growth inhibitory effects of somatostatin and the somatostatin analog on the cholangiocarcinoma cell line.

Thus the results of these two anti-proliferation experiments are depicted graphically in Figures 1 and 2 (Figure 1 graphs the results of the first experiment and Figure 2 graphs the results of the second experiment). The X-axis of the figures depicts time in days. The Y axis represents the percentage of the treated cell count to the control cell count. The two figures demonstrate that both somatostatin and octreotide are effective in inhibiting the growth of the human cholangiocarcinoma cell line. The observed non-dose dependency of both somatostatin and octreotide is inherent with this peptide series. See Setyono-Han, Cancer Research 47:1566(1987) which demonstrates a lack of dose dependency for octreotide in MCF-7 breast cancer cells.

Claims

1. The use of somatostatin or a somatostatin agonist for the manufacture of a medicament for use in a method of inhibiting proliferation of bile duct epithelium.

2. Use as claimed in claim 1 for the manufacture of medicament for use in inhibiting benign tumor growth.

3. Use as claimed in claim 1 for the manufacture of medicament for use in inhibiting malignant tumor growth.

4. Use as claimed in any one of claims 1 to 3 for the manufacture of a medicament for contacting said epithelium with said somatostatin or somatostatin agonist.

5. Use according to any one of claims 1 to 4 for the manufacture of a medicament adapted for parenteral administration.

6. Use according to any one of claims 1 to 4 for the manufacture of a medicament adapted for intravenous administration.

7. Use according to any one of claims 1 to 4 for the manufacture of a medicament adapted for subcutaneous administration.

8. Use according to any one of claims 1 to 4 for the manufacture of a medicament adapted for implantation.

9. Use according to any one of claims 1 to 4 for the manufacture of a medicament adapted for perfusion administration.

10. Use as claimed in any one of claims 1 to 9 of a somatostatin agonist.

11. Use as claimed in claim 10 of an octapeptide somatostatin analog.

12. Use as claimed in claim 10 of octreotide.

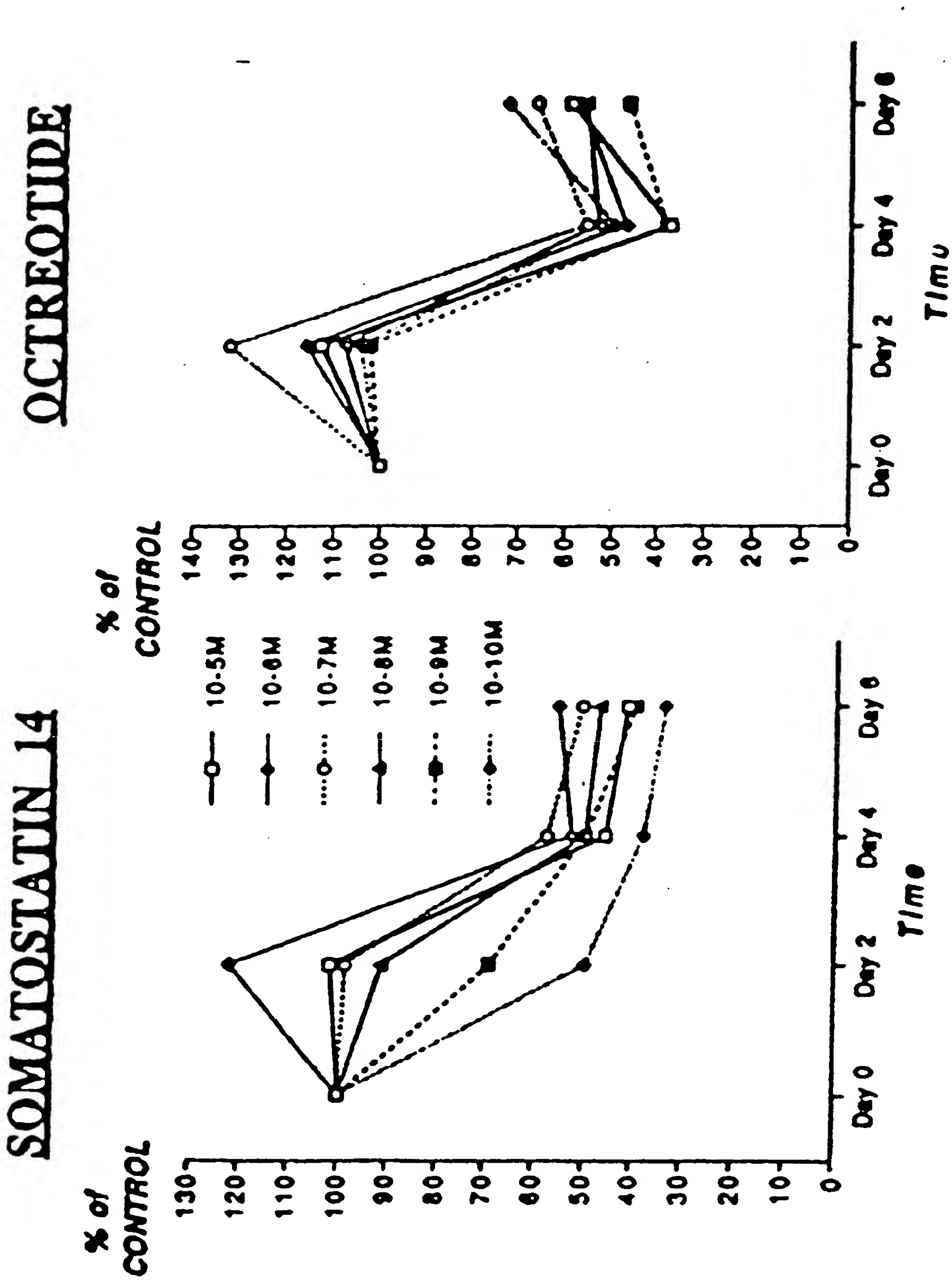
SOMATOSTATIN 14
% of CONTROL
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Day 0
Day 2
Day 4
Day 6
Time
10-5M
10-6M
10-7M
10-8M
10-9M
10-10M
OCTREOTIDE
% of CONTROL
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Day 0
Day 2
Day 4
Day 6
Time

FIGURE 1

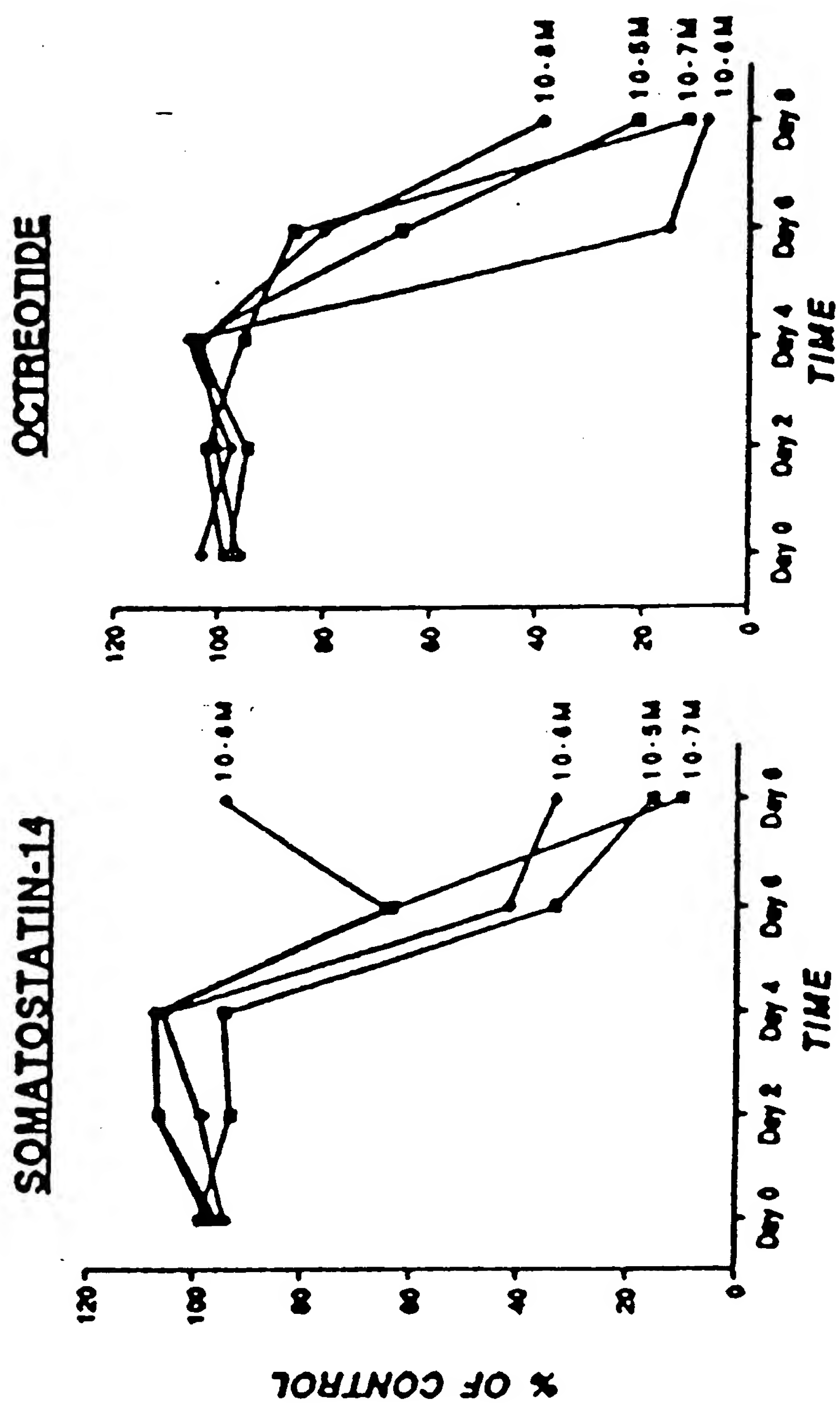
SOMATOSTATIN-14
OCTREOTIDE
% OF CONTROL
TIME
Day 0
Day 2
Day 4
Day 6
Day 8
0
20
40
60
80
100
120
10-8M
10-7M
10-6M
10-5M

FIGURE 2

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 9022

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	INTERNATIONAL JOURNAL OF PANCREATOLOGY, vol.4, no.2, 1989 pages 149 - 160 ZALATNAI A. ET AL. * the whole document *	1-12	A61K38/31
A	GB-A-2 241 167 (SANDOZ) * the whole document *	1-12	
A	EP-A-0 450 480 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) * the whole document *	1-12	
X	THE AMERICAN JOURNAL OF PATHOLOGY, vol.143, no.6, December 1993 pages 1574 - 1578 TRACY T.F. ET AL. * the whole document *	1-12	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			A61K C07K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 March 1995	Moreau, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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